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Cell Wall Regeneration in Woody Protoplasts*¹

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Introduction

In these days, many people use various protoplasts for improvement of plant species because establishment of inducing protoplasts from plants is prerequisite for genetic engineering of woody plant through somatic cell fusion, transformation by electroporation or microinjection. Furthermore as for cell wall regeneration, protoplasts may be also one of the most important materials when we investigate the cell wall or regenerating process of the cell wall. It has been generally accepted that most of woody protoplasts grow slower than most herbaceous one. However, it has not been clarified yet why woody protoplasts grow rather slowly.

In this study, we aimed to investigate cell wall regeneration of woody protoplasts which have little studied in ultrastructural level. Then, the regenerated cell-wall is compared with protoplast-derived wall in tobacco (herbaceous plants). The distribution of xyloglucan in regenerated wall is also investigated using immunogold techniques because xyloglucan is second important structural component in the cell wall of dicot plants.

Materials and methods

Donor tissue and protoplasts culture

Mesophyll tissue in *Populus alba* L. (white poplar) and *Nicotiana tabacum* cv. Xanthi (tobacco), and cotyledons at *Chamaecyparis obtusa* Endl. (Hinoki cypress) were used for donor tissue of protoplasts. (Sasamoto et al. 1992)

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Rapid-freezing and deep-etching

Specimens after a fixation in 3% glutaraldehyde were gradually removed into distilled water. Rapid-freezing of specimens were carried out by contact with a copper block that had been cooled in a liquid helium. The specimens were fractured at -150°C and etched for 15 min at -95°C using a freeze-fracture unit (BAF 400D; Balzers, Liechtenstein), and then rotary-shadowed at 25° with platinum/carbon and coated at 85° with carbon. Subsequently replicas were cleaned in 50% sulfuric acid included with 5% potassium dichromate, washed with distilled water and picked up on copper grids coated with formvar.

Immunogold labelling

Ultrathin sections cut from the samples embedded in LR White resin were treated for 1h in anti-XGO7 (heptasaccharide of xyloglucan) polyclonal antibody and anti-XGO9 (nonasaccharide of xyloglucan) polyclonal antibody, respectively (Sone et al. 1989). After washing in phosphate buffered saline, the grids were incubated for 1h in protein A mixed with 15 nm colloidal gold particle.

Results and Discussion

The regeneration of cell walls in both woody (white poplar and Hinoki cypress) and herbaceous protoplasts (tobacco) was compared in ultrastructural level. Chloroplasts in donor tissue of all three species had ellipsoidal body, respectively. After culturing poplar protoplasts in 24 well plates, chloroplasts were gradually reformed to spherical body within 3 days. Most of chloroplasts in colonies of white poplar and tobacco except for the cypress in which many starch grains are included. In early stage of the cell wall regeneration (10d), the wall compositions distributed sporadically on surface of protoplast-derived cells when the ultrathin sections were observed. However, it was observed that these wall structures consisted of extreme large pores after observation by 3-dimensional images using rapid-freezing and deep-etching techniques. Furthermore, it was also observed by these images that only thin lamella of dense microfibrils existed on the exoplasmic surface. Thin walls were formed after cell division (20d). But the wall structure in white poplar remained loose. When the colony was formed (30d and more), the wall components deposited more densely than before. The structural changes during regeneration of the cell wall were more or less the same with white poplar. In summary, the cell-wall components sporadically deposit on the surface of protoplasts in undivided cells, and perhaps dense microfibril layer exists on the exoplasmic surface of the plasma membrane (Fig. 1). The wall thickness in divided cells increased more than that in undivided cells of all three species, but the wall components in the divided cells did not deposit as much as in colony cells. The wall thickness of the colony cells was similar to that of leaf cells (ca. 200–300 nm) and the network structures of the cell wall possibly possesses large pores.

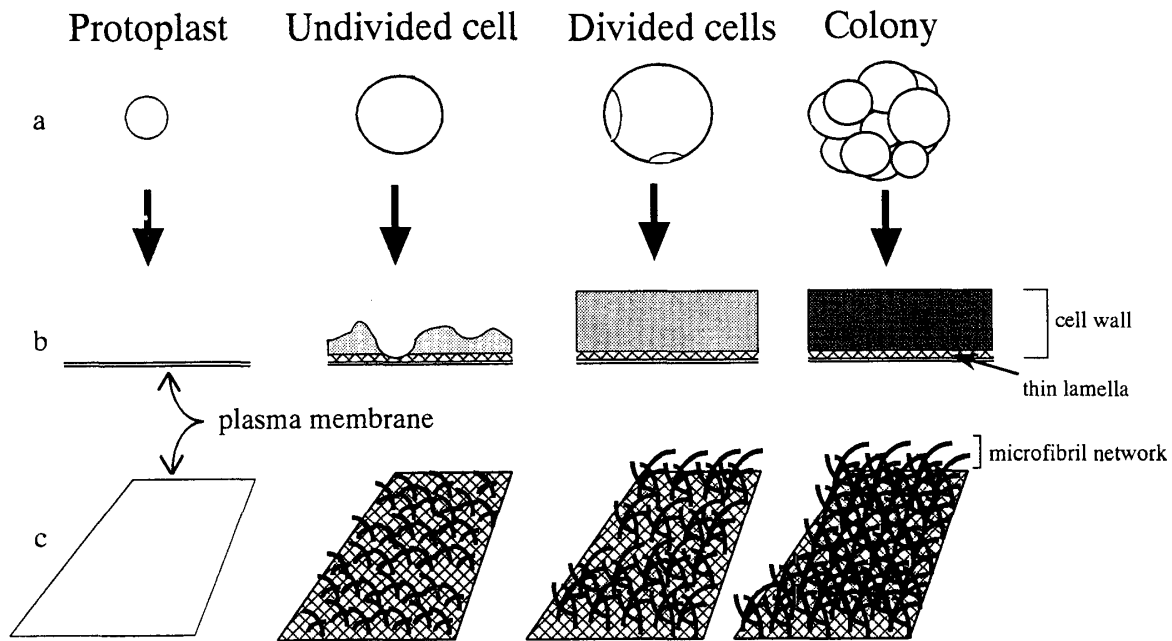


Fig. 1. Schematic representation of wall regeneration from protoplasts. Isolated protoplasts or protoplast-derived cells in different stages are shown in **a**. The cells indicates morphological variation. Cell wall thickness and density observed in ultrathin section are represented in **b**. The 3-D images of the wall structure in rapid-freeze and deep-etch are represented in **c**. The wall network structures in protoplast-derived cells are shown in advanced stages. Thin lamella of dense microfibrils also exists on the exoplasmic surface. Thin lamella on the exoplasmic surface.

What is the chemical components of the network structures other than cellulose? In order to know the answer, the distribution of xyloglucan (XG) in regenerating cell wall was investigated in white poplar and tobacco using immunogold techniques. The xyloglucan is the principal component in the cell wall of dicot plants. As a results, a few gold-particles indicating the site of XG distributed randomly in the early stage (10d). This suggests that XG has already deposited in this stage. In colony cells, the increasing gold-particles distributed randomly in the cell walls. The gold-particles, however, hardly distributed in the cell corner. The observation of regenerating cell wall by deep-etching technique combined with immunogold labelling would be the best way to reconstruct the three dimensional architecture of this nascent wall.

These results were similar in both white poplar and tobacco. It should be noted that the cell-wall structure, cell shape and XG distribution in each stages are more or less the same between the herbaceous and woody protoplast-derived cells, in which 'lag periods' of wall deposition and times to form the colony did not change between these two cell origins. It could be expected that the slower growth in woody protoplasts may be dependent on demand of longer time for the initiation of wall formation.

References

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